Enzo

BioArray™ HighYield™ RNA Transcript Labeling Ki

10 labeling reactions

Distributed by Affymetrix, Inc.

Part No. 900182

For Research Use Only

INTRODUCTION

The ENZO ***BioArray™ HighYield™* RNA Transcript Labeling Kit** has been developed for the production of large amounts of hybridizable biotin-labeled RNA targets by *in vitro* transcription from bacteriophage T7 RNA polymerase promoters. Using T7 RNA polymerase and biotin-labeled nucleotides, large amounts of single stranded nonradioactive RNA molecules can be produced *in vitro*. Because of the nature of transcription reactions, many RNA copies of the template DNA are produced during a short incubation. Because RNA-DNA hybrids have a higher melting temperature than corresponding DNA-DNA hybrids, single-stranded RNA targets offer higher target avidity and greater sensitivity than DNA probes. RNA targets offer selectivity unavailable with DNA targets–being single stranded, they are strand-specific and hybridize more effectively to probes because the target population does not self-hybridize.

RNA transcripts that are labeled with biotin-modified ribonucleotides are used effectively in microchip array assays. The biotin-labeled RNA targets that are hybridized to arrays of DNA probes can be detected by a reporter molecule linked to streptavidin, avidin or anti-biotin antibody. Such a complex can be detected directly, *e.g.*, by excitation of a fluorophore conjugated to streptavidin, or indirectly, *e.g.*, using an enzyme conjugate that can produce an insoluble colored precipitate.

The ENZO ***BioArray™ HighYield™* RNA Transcript Labeling Kit** has been formulated and optimized for use with Affymetrix GeneChip® assays. The kit contains all of the reagents required to perform 10 reactions with approximately 1 µg of transcribable cDNA template such as the product of the Affymetrix Expression Analysis Technical Manual sample preparation protocols, or a control such as plasmid template DNA (0.4 pmol).

REAGENTS PROVIDED

10X HY Reaction Buffer, 40 µl

10X DTT, 40 µl

10X Biotin Labeled Ribonucleotides, 40 µl
ATP, GTP, CTP, UTP with Bio-UTP and Bio-CTP

20X T7 RNA Polymerase, 20 µl
in Storage Buffer

10X RNase Inhibitor Mix, 40 µl
in Storage Buffer with enhancer

EQUIPMENT AND REAGENTS REQUIRED BUT NO PROVIDED

Preparation of RNA Transcripts

- Water bath or heat block set to 37°C
- DEPC-treated, sterile, deionized water

STORAGE

Store all reagents at -20°C, in a freezer that is not self-defrosting.

RNA LABELING

When preparing RNA for use with **Affymetrix GeneChip Expression Arrays**, please refer to the *Affymetrix Expression Analysis Technical Manua*, Chapter 3, "Synthesis of Biotin-Labeled cRNA (IVT)" and Chapter 4, "Control cRNA Preparation".

A. Template Preparation

For control plasmid templates, linearize the plasmid DNA using appropriate restriction enzyme digestion of the template.

- Linearized template DNA should be purified before adding to the reaction.
- Restiction enzymes that leave a 3' overhang should be avoided because T7 RNA polymerase may transcribe these in a promoter independent manner.
- Use only RNase-free water, buffers and pipette tips.

B. RNA Transcript Labeling Reaction

1. Add reaction components to RNase-free microfuge tubes.
2. Make additions in the order indicated in the following table.

NOTE: Keep reactions at room temperature while additions are made to avoid precipitation of DTT.

Reagent	Volume
Template DNA	variable to give l µg of cDNA or 0.4 pmol (about 1 µg of a 3.8 kb plasmid)
Distilled or deionized water	variable (to give a final reaction volume of 40 µl)
10X HY Reaction Buffer	4 µl
10X Biotin Labeled Ribonucleotides	4 µl
10X DTT	4 µl
10X RNase Inhibitor Mix	4 µl
20X T7 RNA Polymerase	2 µl
Total Volume	**40 µl**

3. Carefully mix the reagents and collect the mixture in the bottom of the tube by brief (5 second) microcentrifugation.
4. Immediately place the tube in a 37°C water bath. Incubate for 4 to 5 hours, gently mixing the contents of the tube every 30-45 minutes during the incubation. Longer reaction may increase yield, but the possibility of degradation by RNase increases.
5. Approximately 50-100 µg of RNA product are usually produced in each standard reaction (40 µl reaction containing 1 µg of template DNA). Larger amounts of products can be produced by scaling up all components and volumes. In most scale-up syntheses, the amount of DNA template can be reduced to 0.5-0.8 µg per 40 µl of reaction mixture.
6. Store at -20°C if not purifying RNA immediately.

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PURIFICATION OF LABELED RNA TRANSCRIPTS

We recommend RNeasy spin columns from QIAGEN for purification of labeled RNA.

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For Technical Assistance call ENZO:
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900182-00-9812
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Rev. 1

BioArray™
Terminal Labeling Kit with Biotin-ddUTP for DNA Probe Array Assays

25 labeling reactions

Distributed by Affymetrix, Inc.

Part No. 900181 Rev. 1

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INTRODUCTION

The ENZO ***BioArray*™ Terminal Labeling Kit (with Biotin-ddUTP) for Affymetrix GeneChip® DNA Probe Arrays** has been developed by Enzo specifically for use with the array. The method of labeling uses biotin-ddUTP and terminal deoxynucleotid transferase to catalyze the addition of a single biotin ddUMP to the 3'-OH terminus of the amplified and fragmented target DNA. A standard reaction will label up to 100 picomoles (1 µg of a 30-nucleotide sequence). When DNA fragments are labeled at the 3'-OH terminus, the sequence bias that occurs with either nick translation or random priming is eliminated.

This labeling kit has been formulated and optimized for use with Affymetrix GeneChip® assays.

REAGENTS PROVIDED

The ENZO ***BioArray*™ Terminal Labeling Kit (with Biotin-ddUTP) for Affymetrix DNA Probe Arrays** contains sufficient reagents for 25 labeling reactions of 100 picomoles of fragmented DNA, corresponding to 25 x 1µg of a 30-nucleotide sequence.

Biotin-ddUTP (100X), 25 µl

5X Reaction Buffer, 500 µl
Potassium cacodylate buffer, pH 7, containing β-mercaptoethanol and stabilizer

10X $CoCl_2$ Solution, 250 µl

Terminal Deoxynucleotide Transferase (50X), 50 µl
in storage buffer

WARNING: *The **5X Reaction Buffer** and **Terminal Deoxynucleotide Transferase** contain potassium cacodylate which contains arsenic. It is toxic. Use gloves during the handling of these reagents. Dispose of these reagents and the individual labeling reaction waste materials according to local regulations.*

REAGENTS AND MATERIALS REQUIRED

Preparation and Analysis of Labeled DNA Fragments
- 37°C Water Bath
- Agarose gel (*e.g.*, 4%) - optional
- UV Transilluminator- optional

Termination of Labeling Reaction
- 0.2 M EDTA, pH 8

STORAGE

Upon receipt, store all reagents at -20 °C, in a freezer that is not self defrosting.

PROCEDURE FOR TARGET LABELING

Refer to Affymetrix package insert for target amplification and fragmentation procedures.

1. Add the following reagents (in the indicated order) to a microcentrifuge tube, keeping the tube at **room temperature** while additions are made:

Reagent	Volume
5X Reaction Buffer	20 µl
10X $CoCl_2$	10 µl
Amplified and fragmented target	variable (as specified in Affymetrix target amplification and fragmentation package insert)
Biotin-ddUTP	1 µl
Terminal Deoxynucleotide Transferase	2 µl
Distilled or deionized water	variable (to give a final reaction volume of 100 µl)
Total Volume	**100 µl**

2. Carefully mix the reagents in the tube and collect the mixture in the bottom of the microcentrifuge tube by brief (5 seconds) microcentrifugation.
3. Incubate the tube for **15** minutes in a 37 °C water bath. The reaction may be allowed to proceed for up to 1 hour.
4. If desired, transfer the tube to ice water (2-4°C), remove 2 µl and analyze on an agarose or acrylamide gel.
5. Stop the reaction by adding 5 µl of 0.2 M EDTA.
6. Store the labeled DNA fragments at -20°C. Do not freeze and thaw repeatedly.

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